INTERNATIONAL STANDARD

ISO 5552

Second edition
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Meat and meat products — Detection and enumeration of *Enterobacteriaceae* without resuscitation — MPN technique and colony-count technique

Viande et produits à base de viande — Recherche et dénombrement des Enterobacteriaceae sans ressuscitation — Technique de NPP et technique de comptage de colonies

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Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

This second edition cancels and replaces the first edition (ISO 5552:1979), which has been technically revised.

International Standard ISO 5552 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products*.

Annexes A and B form an integral part of this International Standard. Annex C is for information only.

Introduction

During a meeting in March 1996 of Subcommittees SC 5, *Milk and milk products*, SC 6, *Meat and meat products*, and SC 9, *Microbiology*, it was recommended to put forward one horizontal standard for the detection and enumeration of *Enterobacteriaceae*. This needs a revision of the two horizontal standards ISO 7402 and ISO 8523 into one standard, divided into three parts.

ISO 5552 will be withdrawn as soon as the combined horizontal method is published.

Meat and meat products — Detection and enumeration of *Enterobacteriaceae* without resuscitation — MPN technique and colony-count technique

1 Scope

This International Standard specifies a method for the detection and enumeration of *Enterobacteriaceae* present in all kinds of meat and meat products, including poultry. Enumeration is carried out as follows:

– by calculation of the most probable number (MPN) after incubation at 35 °C or 37 °C in liquid medium; or

– by counting colonies in a solid medium after incubation at 35 °C or 37 °C.

The temperature used is to be the subject of agreement between the parties concerned, and is to be stated in the test report.

NOTE In the case of frozen foods, an incubation temperature of 30 °C is preferred when the aim of the enumeration is technological.

For low numbers, the MPN method is preferable, otherwise the colony-count method is preferred.

This International Standard does not include resuscitation procedures and the results should not, therefore, be related to criteria or specifications based on the assumption that resuscitation has been carried out.

A limitation on the applicability of this International Standard is imposed by susceptibility of the methods to a large degree of variability. The methods should be used and the results interpreted in the light of the information given in 10.3.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3100-2:1988, *Meat and meat products — Sampling and preparation of test samples — Part 2: Preparation of test samples for microbiological examination.*

ISO 6887:1983, *Microbiology of food and animal feeding stuffs — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

ISO 7402:1993, *Microbiology — General guidance for the enumeration of Enterobacteriaceae without resuscitation — MPN technique and colony-count technique.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 *Enterobacteriaceae*
Microorganisms which ferment glucose and show a negative oxidase reaction when the test is carried out according to the method specified.

3.2 detection of *Enterobacteriaceae*
Determination of the presence or absence of these bacteria, in a particular mass of product, when tests are carried out in accordance with this International Standard.

3.3 count of *Enterobacteriaceae*
Number of *Enterobacteriaceae* found per millilitre or per gram of the test sample when the test is carried out according to the method specified.

4 Principle

4.1 Preparation of dilutions

Preparation of decimal dilutions from the test sample.

4.2 Detection of *Enterobacteriaceae* in a specified quantity of sample

Introduction of 1 ml of the test sample if the product is liquid or of the initial suspension in the case of other products (or of decimal dilutions of these), into a tube containing a selective enrichment broth.

Incubation of the tubes at 35 °C or 37 °C for 24 h, followed by streaking of the cultures onto violet red bile glucose agar. After incubation of the streaked agar plates at 35 °C or 37 °C for 24 h, subjection of suspected colonies to biochemical confirmation tests.

4.3 Enumeration of *Enterobacteriaceae*

4.3.1 Most probable number (MPN) technique

NOTE This technique is recommended when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of the test sample.

Inoculation of three tubes of double-strength medium with a specified quantity of test sample if the product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Inoculation of three tubes of single-strength medium with a specified quantity of the test sample if the product is liquid, or with a specified quantity of the initial suspension in the case of other products. Then under the same conditions, inoculation of three tubes of single-strength medium with the first decimal dilution of the test sample or of the initial suspension.

Incubation of the tubes at 35 °C or 37 °C (as agreed) for 24 h.

From the number of confirmed positive tubes, calculation of the most probable number of *Enterobacteriaceae* per millilitre or per gram of the test sample using the MPN table (see annex A).

4.3.2 Colony-count technique

NOTE This technique is recommended when the number sought is expected to be greater than 100 per millilitre or per gram of the test sample.

Inoculation of violet red bile glucose agar contained in two Petri dishes (poured-plate technique) with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products. Covering with an overlayer of the same medium.

Preparation of other pairs of plates under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

Incubation of the dishes at 35 °C or 37 °C (as agreed) for 24 h ± 2 h.

Calculation of the number of *Enterobacteriaceae* per millilitre or per gram of the test sample from the number of confirmed typical colonies per dish.

5 Diluent, culture media and reagent

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Diluent

See ISO 6887.

5.3 Culture media

5.3.1 Buffered brilliant green bile glucose broth (EE broth)

5.3.1.1 Composition

	a) Double-strength medium	b) Single-strength medium
Peptone	20,0 g	10,0 g
Glucose	10,0 g	5,0 g
Disodium hydrogen phosphate (Na_2HPO_4)	12,90 g	6,45 g
Potassium dihydrogen phosphate (KH_2PO_4)	4,0 g	2,0 g
Ox bile, dehydrated	40,0 g	20,0 g
Brilliant green	0,027 g	0,0135 g
Water	1 000 ml	1 000 ml

5.3.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling. Adjust the pH, if necessary, so that after boiling it is 7,2 at 25 °C. Do not heat the medium for longer than 30 min. Cool the medium rapidly.

Aseptically transfer 10 ml portions to sterile tubes or bottles (6.8).

Do not autoclave the medium.

The medium may be stored for up to 1 week at between 0 °C and 5 °C.

5.3.2 Violet red bile glucose agar (VRBG)

5.3.2.1 Composition

Peptone	7,0 g
Yeast extract	3,0 g
Bile salts	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Neutral red	0,03 g
Crystal violet	0,002 g
Agar in powder or flake form	8 g to 18 g[1)]
Water	1 000 ml

1) Depending on the gel strength of the agar.

5.3.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling. The medium must not be boiled for more than 2 min.

Adjust the pH, if necessary, so that after boiling it is 7,4 at 25 °C.

Transfer the culture medium to sterile tubes, flasks or bottles (6.8) of capacity not more than 500 ml.

Do not autoclave the medium.

Prepare this medium just before use (see 9.3.2 and 9.4.1).

5.3.2.3 Preparation of agar plates (required for detection and MPN technique, see 9.3.2)

Dispense immediately approximately 15 ml of the culture medium, cooled to approximately 47 °C in the water bath (6.5), into Petri dishes (6.6) and allow to solidify.

Immediately before use, dry the plates, preferably with the lids off and the agar surface downwards, in the oven (6.3) until the agar surface is dry.

If prepared in advance, the undried plates shall not be kept for longer than 4 days at between 0 °C and 5 °C.

5.3.3 Glucose agar

5.3.31 Composition

Tryptone	10,0 g
Yeast extract	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Bromocresol purple	0,015 g
Agar in powder or flake form	8 g to 18 g[1)]
Water	1 000 ml
1) Depending on the gel strength of the agar.	

5.3.3.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,0 at 25 °C.

Dispense the culture medium in quantities of 15 ml into tubes or flasks (6.8).

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

Leave the tubes or flasks in a vertical position.

The medium may be stored for up to 1 week at between 0 °C and 5 °C.

Just before use, heat in boiling water or flowing steam for 15 min, then cool rapidly to the incubation temperature.

5.3.4 Nutrient agar

5.3.4.1 Composition

Beef extract	3,0 g
Peptone	5,0 g
Agar in powder or flake form	8 g to 18 g[1)]
Water	1 000 ml
1) Depending on the gel strength of the agar.	

5.3.4.2 Preparation

Dissolve the components or dehydrated complete medium in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,0 at 25 °C.

Transfer the culture medium to tubes, bottles or flasks (6.8) of capacity not more than 500 ml.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.